PITUITARY IMPLANTS AND THE REPRODUCTION CYCLE OF THE THIRTEEN LINED GROUND SQUIRREL

by

MARK ANTHONY FOSTER

A. B., Louisiana State Normal College, 1929

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE

OF AGRICULTURE AND APPLIED SCIENCE

1931

378.73
Docu-
ment
LD
2668
.T4
1931
F61
c.2

TABLE OF CONTENTS

page

INTRODUCTION 3

REVIEW OF LITERATURE 3

METHODS AND MATERIALS 9

General 9
Histological Technique 10
Implantation 10
Vaginal Smears 12
Hibernation 12

SEXUAL CYCLE OF THE LABORATORY FEMALE 13

The Anoestrous Period (July to December, inclusive) 13
The Period from January to June 17

SEXUAL ACTIVITY OF THE LABORATORY MALES 22

The Inactive Period 22
The Active Period 22

OBSERVATIONS ON NORMAL OUTDOOR ANIMALS 26

Females 26
Males 28

THE EFFECT OF PITUITARY IMPLANTS 28

DISCUSSION 32

CONCLUSIONS 35

ACKNOWLEDGEMENTS 36

LITERATURE CITED 40

INTRODUCTION

It has been known for many years that a number of wild animals do not reproduce normally in captivity. In this group of animals is the thirteen-lined ground squirrel, Citellus tridecemlineatus. During the last seven years there has been an average of over 100 animals in the animal house at the beginning of the breeding season each year, and of this entire group we have proof of only five litters.

Previous work in this laboratory on the cause of this lack of reproduction has shown the need for a complete study of the reproductive cycle. In this paper will be reported further studies on the reproductive cycle of this animal under laboratory conditions, and the results of pituitary gland implants made through the entire year.

REVIEW OF LITERATURE

Lee (1902) observed that male ground-squirrels of the typical variety, Citellus tridecemlineatus (Mitchill), made their appearance soon after they ceased hibernating, usually between April 10 and 25 in Minnesota.

The breeding season follows shortly upon their awakening in the spring (Johnson, 1917; Dripe, 1919;

and O. Wade, 1927). An inactivity of the ovary during the fall and winter was observed by Dripe (1919), who found that ovulation occurs once a year following copulation. Wade (1927) considers the rutting season commonly lasts two weeks, but under certain conditions may last as long as four weeks.

The gestation period of the typical variety was found to be about 28 days by Dripe (1919), and between 27 and 28 days by O. Wade, (1927) in three cases of copulation observed among recently captured animals. The gestation period in the paler variety ~~C. t. pallidus~~ was observed to be about 27.5 days in one female seen in copulation in this laboratory in 1927. No cases of reproduction among ground squirrels which have been in the laboratory over winter are on record (Johnson and Wade, 1931).

Male ground-squirrels appear before the females in the spring as shown by Shaw (1925) for the Columbian ground squirrel, and by Lee (1902) and Wade (1927) for the thirteen-lined form.

Various factors may have a bearing on the reproductive activities of wild animals. Shaw (1925) found that food, locality and length of season affected the Columbian ground squirrel in nature. Dice (1929) found fighting inhibited reproduction in cottontail rabbits,

Marshall (1922) showed that captivity interferes with breeding of many wild animals besides rodents.

It has been generally recognized that fatness is a cause of sterility among domesticated animals (Marshall and Peel, 1910), but whether this has a relationship to the pituitary body is not known. Diminished food supply and low temperature are considered as causes of the prolonged anoestrous of wild rodents in the winter by Parkes and Bramwell (1927).

Laboratory rodents do not give much insight into reproduction in the ground-squirrel. The guinea pig has an average oestrous cycle of 16 days, and oestrous lasts about 24 hours. It is accompanied by certain changes in the vaginal epithelium (Stockard and Papanicolaou, 1917, 1919; and Selle, 1922). Long and Evans (1922) found the rat to have an average cycle of about four days with a characteristic vaginal smear for each phase of the cycle, and E. Allen (1922) found the mouse to have a four to six day cycle with a similar characteristic vaginal fluid for each phase of the cycle. It may be added that the vaginal smear method has been found valuable also in the study of the phases of the 28-day cycle of the opossum (Hartman, 1923), and of the cycle of the dog (Evans and Cole, 1928).

The reproduction cycle of the ferret appears to resemble that of the ground squirrel more than do those of laboratory rodents (Marshall, 1904). It has a long anoestrous period, the vulva enlarges in oestrus, ovulation takes place only after coitus, and heat is prolonged in the absence of coitus (Hammond and Marshall, 1930).

From the literature it is evident that oestrus is generally produced by a hormone produced chiefly in the graafian follicle (Allen and Doisey, 1923; Marshall and Wood, 1923; Asdell and Marshall, 1927; Papanicolaou and Blau, 1927; Bramwell and Parkes, 1927; and others). Follicular development is in turn dependent upon the secretion of the anterior lobe of the hypophysis as shown in various animals by the implant method (P.E. Smith, 1926, 1927; Smith and Engle, 1927; Zondek and Aschheim, 1927; E. Allen, 1928; Wolf, 1929; Hartman, 1930; Johnson and Wade, 1931). Further evidence in this direction was produced by injecting an extract of the urine of pregnant women, which apparently contains the maturity-producing hormone of the anterior pituitary (Zondek and Aschheim, 1928), but this was found to be less effective than the implantations of the whole gland (Evans and Simpson, 1929).

A lipoid extract of the pituitary also stimulated follicle formation (Brouha and Simonnet, 1927) as did also

acid extracts (Cushing and Teel, 1929). Hill and Parkes (1930) also prepared an active extract which produced in the anoestrous ferret an oestrous condition in about 8-10 days.

The corpus luteum has been found to be essential to the maintenance of pregnancy in a number of species (Corner, 1928; Parkes, 1928; Harris and Pfiffner, 1929; Johnson and Challans, 1931, and others). It has also an oestrus-inhibiting function as found by Parkes and Bellerby, (1927). The activity of the corpus luteum may apparently normally stop before parturition (Courrier and Kehl, 1929), and continued administration of a corpus luteum extract may delay parturition by days (Nelson, Pfiffner and Haterius, 1930; Johnson and Challans, 1931). That the corpus luteum also depends upon the anterior pituitary is shown by injections of an alkaline extract of the latter (Evans and Long, 1922; Johnson and Saylee, 1929; Evans and Simpson, 1927; Johnson and Hill, 1930; Lepine, 1931). Marrian and Parkes (1929) produced oestrus with anterior pituitary substance in animals in anoestrous as a result of a vitamin B free diet.

In male animals it is evident that the prostate, the seminal vesicle, Cowper's gland and the vas deferens are maintained in functional activity by a secretion from the

from the testes (Moore and Gallagher, 1930). The testes, however, do not seem to be stimulated by pituitary implants to the extent to which the ovaries are (Smith and Engle, 1927; Johnson and Hill, 1930; Johnson and Wade, 1931; Hill and Parkes, 1930).

Besides influencing reproduction the anterior pituitary gland promotes growth (Evans and Long, 1921, 1922; Johnson and Sayles, 1929; Evans and Simpson, 1929; Putnam, Benedict and Teel, 1929; Johnson and Hill, 1930; Smith, 1930), and may cause acromegaly (Putnam, Benedict and Teel, 1929; Teel, 1929).

Some success in the separation of the growth-producing, the luteinizing and the maturity-producing hormones of the anterior pituitary have been made by Putnam (1929), Fevold, Hisaw and Leonard, (1930, 1931), and Claus (1931).

Evidence that the stimulating effect of the anterior pituitary upon the reproductive organs may be inhibited by large amounts of either male or female sex hormone obtained from testes and follicles, respectively, has been presented by Moore and Price (1930), and Meyer, Leonard, Hisaw and Martin (1930).

These workers and also Engle (1929) have presented evidence that this inhibitory action of the sex hormones has been responsible for the idea that there is an antagonism between the testes and the ovary when either one has

been engrafted into the body of an animal of the opposite sex.

METHODS AND MATERIALS

General

Ground squirrels from western Kansas which had been in the animal house for several months to over a year were used in this work. They were either Citellus tridecemlineatus pallidus(Allen) or the new variety which Howell has separated from this variety, viz. C. t. arenicola. The animals were smaller and paler than the typical variety of eastern Kansas. The animals were fed an adequate diet and were in good condition. The cages were large and contained nest and bedding materials. The temperature was usually about 70 - 85 deg. F. so very few of the animals hibernated in this room.

In the study of the reproductive cycle of these animals observations at intervals of a few days were made throughout the year. Unusually fat or thin animals were not considered as normal. In the males the enlargement and descent of the testes into the scrotum was watched. When the testes were not felt and there was no scrotum, the animal was recorded as "not scrotal". When the testes

were slightly enlarged and partially descended into an enlarged scrotum, they were said to be "partly scrotal." Those with enlarged, black scrota, and medium to large testes were considered "scrotal." In describing the sexual condition of the females the terms normal, medium, and enlarged vulva were used. If the vagina happened to be open, this was also stated.

Histological Technique

During the year both males and females were sacrificed at intervals of several days, and their tissues fixed in various fixing fluids. For the female sex organs Bouin's fixative was almost universally used, while in the case of the males Allen's modification of Bouin's was used in some cases, but for the most part Hance's (1917) modification of Flemming's Strong was used for the testes. Several stains were used. For general staining, Cornhouser's (1930) Hematin Method was used, while for the seminiferous tubules Iron Hematoxylin was used in most cases. Other stains were used experimentally, but those given above proved most satisfactory for this particular problem.

Implantation

As a routine part of the problem, animals were regularly implanted at intervals of one month, and for the

most part the following technique was used: A small piece of the ovary, in the case of the female, (one fourth to one half) was removed before the implanting began to serve as a control piece of tissue for the individual. This was done to eliminate the errors which would have arisen as the result of variation, for it would have been exceedingly difficult to say whether or not one animal was in the same sexual condition as another. An interval of 24 - 48 hrs. was allowed for recovery, and the implants were administered daily for several days. Then the sliced ovary was removed for study of the effects of the implanting for a short period. Another interval was allowed for recovery, and implanting was again resumed until the desired effect had been produced. The remaining ovary was then removed for histological study.

In the males, a similar treatment was used. Instead of breaking open the tunic, however, a sharpened needle was used to open it in a portion which contained no blood vessels. A few of the tubules were then forced out and cut. The testis was replaced in the scrotum, and it was sewed. Normal recovery followed shortly, and implanting was done as in the females.

The implanting of pituitaries was carried on under aseptic conditions at all times, and speed was at the

sons was maintained. One assistant caught the animal, while the other removed the pituitary from the rat, placed it in some Ringer's or Locke's solution and forced it orally into a long-necked capsule. A small incision was made with a needle, and while the animal was held the gland was implanted subcutaneously, usually along the back. The entire process averaged 2 — 3 minutes, and no anaesthetic was used.

Vaginal Smears

During the earlier part of the work, vaginal smears were made only after pituitary implants due to the fact that the vaginae of the females were closed. However, it soon became apparent that various changes in the swellings of the vulva showed characteristic smears. Then, smears were made from all animals with open vaginae to determine if possible the significance of the vaginal fluid. The technique used was that of Long and Evans (1922) for the rat, and eosin-bluish was used for a stain, since it stained the cells instantly.

Hibernation

Animals were placed in the refrigerator at a temperature ranging from 4 to 10°C. Both normal and implanted animals were placed under hibernating conditions

for from two to 10 weeks. Observations were made daily as to their condition, both during and after hibernation as to the general and sexual condition of the animals.

SEXUAL CYCLE OF THE LABORATORY FEMALE

The Anoestrous Period (July to December, inclusive)

The period from July to December, inclusive, may be said to be the anoestrous period of the ground squirrel. It is characterized by a lack of sexual activity (Table I). The vulva is very small, and its lips are fused ventrally, so that mating would be impossible. In general, the females are in good condition during this entire period. The body is surrounded by subcutaneous fat, and large masses may be found in the mesenteries surrounding the kidneys, and in the mesovaria surrounding the ovaries and at the sides of the uteri.

The animals are active during the entire period. In the fall before the animal house is heated they seem to be drowsy after cool nights, but after the steam was turned on they showed little if any tendency to hibernate. The increased tendency to fight in the fall may indicate that animals in the field hibernate singly (Johnson, 1917). Their food requirements are about the same as in the other seasons.

Table I. Observations on Females

Date	Normal	Vagina sl. swollen	Vagina very swollen	Vagina open
1930				
July to				
December	All*	0	0	0
1931				
Jan. 9,	28	0	1	0
Jan. 24,	16	0	10	2
Feb. 1,	12	0	13	3
Feb. 11,	3	13	16(7)	3
Feb. 26,	0	10	15(11)	6**
March 12,	3	5(4)	18(10)	5(2)
March 29,	1	4(3)	14(8)	10(6)
April 10,	1	8	15(8)	5(2)
May 5,	12	6	1(1)	0
May 27,	19	9	0	0
June 20,	26	1	1	0
June 25,	26	0	0	2(1)
July 20,	29	0	0	0

* No genital development was seen in any handling of the animals nor in a number of special observations made of several animals at different times during this period.

** Two litters born on this date from old females.

Numbers in parentheses indicate the number of females received as young in 1930. Included in numbers to the left.

Histological examination of the ovary, Table II, shows a very compact body bounded by cogenia along the periphery. Inside the cogenia are all sizes of follicles, the arrangement being from the smaller to the larger as one moves inward. The larger follicles measure 0.24-0.30 mm., and in many cases are accompanied by atretic follicles. The interstitial cells show a tendency to clump together in many cases, and average 4 micra in diameter.

The uterus during this period is also very small, and is imbedded in the large masses of fat in the mesovaria. It has a pale pinkish color, and averages 1 mm. in diameter. Histologically it has an inactive appearance in so far as the appearance of the cells are concerned. The muscle layers are very thin, as well as the mucosa. The mucosa cells are small and the glands are scarcely visible in many cases, while in others they could not be seen at all. The epithelium was only one or two layers thick, and the lumen was very small.

The vagina was also very small with its walls folded very closely to make a small lumen. It was lined with small epithelial cells, and the mucous and submucous layers were very thin.

The above description is the typical condition for the entire period, except for a general size development which

Table II. Histological Observations on Laboratory Females

Date	Ovary	Uterus	Vagina	Size large follicles in mm.; other data.*
1930				
Sept.				
27	2X1.5	1.0	closed	0.20-0.34; many sm. & atr. foll.
Oct.2	2X1.5	1.0	closed	0.24-0.30; sm., med. & atr. follicles.
28	2X1.5	1.0	closed	0.24-0.30; sm., med. & atr. follicles; uterus atretic, glands not visible, mucosa thin.
Nov.				
6	2.5X2	1.5	closed	0.24-0.40; many eosinia along periphery.
13	2.5X2	2.5	closed	0.30-0.40; med. & sm. follicles, much connective tissue.
Dec.3	2.5X2	2.2	closed	0.32-0.46; sm., med., & atr. follicles.
5	2.5X2	2.0	closed	0.32-0.40; med. & sm. follicles, much connective tissue.
1931				
Jan.6	2X1.5	1.0	closed	0.32-0.36; sm., med. & large follicles.
7	2X1.5	1.0	closed	0.32-0.36; same, eosinia along periphery.
26	3X2	2.0	closed	0.36-0.45; sm., med. & large follicles, many atretic.
Feb.				
14	4X3	3.0	open	0.40-0.52; 3 corpora lutea rt. ov.; sm., med., large & atr. foll.; uterine glands large; vagina lined ep. c.
27	3X2	1.5	closed	0.30-0.37; many atretic; few med. & sm. follicles.
March				
9	3X2	2.3	open	0.30-0.40; many large & atr., few med. & sm. follicles.
31	4X3	2.5	closed	0.36-0.42; 4 corpora lutea; sm., med. & large foll.
April				
11	3X2	Pregnant	open	0.26-0.32; 3 corp. lutea(1.0-1.2), med. & atr. follicles.
14	3X2	3.0	partly open	0.32-0.34; med. & atr. follicles.
June				
3	2X1.5	2.5	slightly swollen	0.30-0.36; many atretic, few medium follicles.
19	3X2	1.0	ing	0.30-0.36; many atretic foll., degenerating corpora lutea.

* Abbreviations used: sm., small; med., medium; atr., atretic; foll., follicles.

gradually manifests itself during the latter part of the period by an evident growth of the follicles and uteri.

The Period from January to June

The first six months of the year include the period in which the laboratory female shows the only indication of becoming sexually active. There is a great amount of individual variation(Table III.). In general, each female passes through the following stages as shown by external changes. First, a period of slight swelling of the vulva which lasts several weeks and passes into a period of marked swelling which lasts for a variable length of time and usually culminates in the opening of the vagina. The vagina remains open for varying periods of time, but usually lasts from 7 to 10 days, and eventually recedes. However, in several cases it evidently opened, closed, and reopened before it finally receded.

These vaginal swellings were very inconspicuous in many cases and were never as marked as in the case of the outdoor ground squirrel. This condition made it very difficult to distinguish between the different stages. Due to the fact that pregnant females had very swollen vaginae which could be opened, they were often described as "very swollen".

Table III. Individual Observations on Females.

Date(1931)	1	2	3	4	5	6	7	8	9	10	11	12
Dec. 20	.*	.	.	.	.	.	.	.	.	.	.	.
Jan. 9	.	.	.	.	.	.	.	.	.	.	.	.
24	O	O	O	Ø	Ø	.	.	.	.	.	.	.
Feb. 1	O	Ø	O	Ø	Ø	Ø	.	.	o	.	O	O
11	Ø	O	o	Ø	Ø	O	.	.	O	o	Ø	Ø
28	Ø	Ø	O	L**	L	O	.	.	Ø	O	O	Ø
March 12	Ø	Ø	O	O	O	D	O	O	Ø	O	O	Ø
29	Ø	D	O	O	O		b	Ø	Ø	O	Ø	O
April 10	O		Ø	O	O		O	O	Ø	O	O	O
May 5	o		o	.	.		.	o	o	.	D	.
27	.		o	.	.		.	.	o	.		.
June 20	.		.	.	.		.	.	.	.		.

* ., indicates normal female; o, slightly swollen vulva; O, very swollen vulva; Ø, open vagina; L**, litter on this date; D, animal sacrificed.

The first female to show any considerable swelling of the vagina was found Jan. 5(not shown on Table III). She was the only laboratory female to show as great a swelling as was found in the normal outdoor female. This female mated, as was shown by corpora lutea(Table II, Feb. 14).

The other females were found with swollen and open vaginae on the dates shown on Table I. The greatest sexual development occurred in February and March, while the known matings were distributed as follows: Three occurred in January and one in March. There was a gradual increase in activity in January and a marked decrease in April. Comparison will be made later with the mating season of the normal outdoor females.

Histologically it will be necessary to consider three stages of development during this period, viz., the stage before the vagina opens, the stage during which the vagina remains open and the receding stage.

The stage before the opening of the vagina is marked by a hypertrophy of the entire genital apparatus. The ovaries show an increase in size due to growth of the follicles. The large follicles increase from about .30mm. to an average of about 0.35mm. Atretic follicles are also present. The cogenic and small follicles which filled the entire periphery during the anoestrous period have undergone a complete transformation. A number have become atretic follicles, while the remaining ones have increased in size and as a result of this growth have been crowded together. In all the stages of development, there seems to be a growth of certain follicles at the expense of others.

The uterus shows a similar enlargement, which seems to be due to a hypertrophy of the cells of all layers to the surface. The glands appear during this stage as large bundles of cells in the mucosa, which show a lumen toward the end of the period. The epithelial lining has increased in thickness from one or two layers to three or four layers.

The vagina shows an enlargement also, and this enlargement corresponds to the condition of the ovaries and uteri. The vulval swellings afford a fairly accurate index to the condition of the ovaries and uteri. During this stage of development every layer of cells one undergoes a marked hypertrophy, and the epithelial lining has increased to a thickness of seven or nine layers of cells.

The open vagina stage probably includes part of the proestrous, estrus, and part of the metaestrus stages, but due to the variation among the animals in the time of coming into these stages this part has been omitted for future work.

The ovaries studied from females with open vaginae were almost twice as large as the normal ovary of anoestrum. They contained from seven to fifteen enlarged follicles which measured from 0.40-0.52mm. Many medium and small follicles were present in some cases. Atretic follicles

atretic follicles were usually abundant, and in a few cases corpora lutea were found.

The uteri were unusually large as compared to the inactive period. They averaged three mm. in diameter, and the fat pads were not so conspicuous. The appearance of the whole organ had changed from a light pink color to a rich, blood-red color due to the enlarged blood vessels. Contractions of the uteri were very noticeable during this period.

Histological examination showed the glands of the uteri to be conjested with a colorless fluid, and the epithelial lining of the vagina to be covered with a layer of flat cornified cells in some cases.

The stage of regression was marked externally by a closing of the vagina, which was accompanied by a decrease in size until it reached the normal anoestrous condition. Accompanying the decrease in size of the ovaries and uteri was an increase in the amount of fat present in the mesenteries and mesovaria. The decrease in the size of the ovaries seemed to be due to a decrease in the size of the follicles, and in a few cases by a disintegration of the corpora lutea.

It is very evident that the corpora lutea are formed from two types of cells as described by Dripa, (1919).

SEXUAL ACTIVITY OF THE LABORATORY MALES

The Inactive Period

In the male ground squirrels there was not such a variation as was found in the females, and the slight variations of individual animals that did occur would be accounted for by the condition of the animal. From the latter part of July until November 15 the males were in a semi-scrotal condition (Table XII). The testes were small, averaging 11X6X5mm. (Table IV) and drawn up into the abdomen. Practically all seminiferous tubules examined during this period showed only spermatogonia and primary spermatocytes, except toward the end of the period.

The Active Period

From November 15 to the latter part of the month there was a noticeable development in the size of the testes of the older males. By the end of December most tubules studied showed all stages of spermatogenesis. The younger males did not show much development before the middle of January, but observations on January 24 showed all males to be "scrotal" on that date with the exception of two males which were undernourished.

Table IV. Observations on Sexuality in Males

Date	No. Normal Males	No. Males ab. sexual	No. Males sexual
Sept. 1	20	0	0
Nov. 15	—	5	0
Dec. 1	10	6	5
Jan. 9	5	0	16
Jan. 24	3	0	25
Feb. 1	1	0	27
Feb. 11	0	1	31
Feb. 25	0	0	34
March 12	0	0	31
March 29	0	0	30
April 10	0	0	30
May 27	3	5	23
June 17	12	6	12
July 1	20	7	3
July 20	26	2	0

Note: Variation in number of males observed was due to the fact that certain animals were taken for experimental purposes.

Preceding the "scrotal" condition there was a great increase in the size of the testes culminating in their descent into the scrotum. The hair on the scrotum gradually came off and it darkened in color.

All scrotal males examined after January 10, until June 1 were found to be in active spermatogenesis. The scrotal condition in the males lasted in most cases until the latter part of May, and some were even scrotal as late as June 10. At this time the animals were noted to be rather fat, and with the increase in subcutaneous fat there was a decrease in the size of the testes. This passing from the scrotal to the non-scrotal condition is characterized by both internal and external changes. Externally, there is first a loss of the dark color on the scrotum. Then there is a growth of hair over the scrotum so that it eventually disappears.

Internally the testes seem to gradually decrease in size, and they are drawn up into the abdomen. The seminiferous tubules seem to decrease in size as the cells mature. That is, the number of spermatogonia seems to remain the same, while the spermatocytes and spermatids mature into spermatozoa and are passed out, thus leaving a seminiferous tubule with a lining of spermatogonia and Sertoli cells.

Table V. Size and Development of Testes

Date	Size Testes (mm.)			Age	Sperm in Vas def.	Stage of Development
1930						
July 11	10X	5X	5	old	none	Spermatogonia
July 12	12X	6X	6	old	none	Spermatogonia
Aug. 9	10X	6X	6*	old	none	Spermatogonia
Sept. 23	7X	5X	5	young	none	Spermatogonia few Pr.Sp.
Sept. 27	12X	7X	6	old	some	Primary Spermatocytes
Sept. 28	14X	8X	7	old	none	Primary Spermatocytes
Oct. 2	8X	7X	5	young	none	Few Pr. Spermatocytes
Oct. 30	10X	9X	7	young	none	Few Pr. Spermatocytes
Dec. 6	13X	6X	5	old	some	Few Sec. Spermatocytes
Dec. 6	13X	8X	6	old	none	Primary Spermatocytes
Dec. 20	14X	7X	5	old	none	Sec. Spermatocytes
Dec. 29	17X	7X	5	old	yes	All Stages, Spermatozoa
1931						
Jan. 6	19X	11X	10	old	yes	All Stages, Spermatozoa
Jan. 10	10X	6X	4	young	none	Spermatids
Jan. 26	20X	10X	8	old	yes	All Stages, Spermatozoa
Jan. 27	19X	11X	10	old	yes	All Stages, Spermatozoa
Feb. 14	14X	7X	6	young	yes	All Stages, Spermatozoa
Feb. 24	21X	12X	10	old	yes	All Stages, Spermatozoa
Feb. 26	20X	10X	8	old	yes	All Stages, Spermatozoa
April 14	18X	16X	10	old	yes	All Stages, Spermatozoa
May 30	18X	9X	7	old	yes	All Stages, Spermatozoa
June 8	12X	8X	5	old	yes	Spermatozoa
June 11	15X	9X	8	old	none	Spermatozoa
June 14	10X	5X	4	old	none	No Data
June 15	10X	5X	4	old	none	No Data
July 25	8X	4X	3	old	none	No Data

Note: Old animals are those that have been in the laboratory for one year or longer (1930 or earlier). Young animals are animals born in the spring of 1930.

* This data was supplied through the courtesy of E. Fado.

OBSERVATIONS ON NORMAL OUTDOOR ANIMALS

Females

The first normal outdoor animals received from the field were delivered to the laboratory April 16. Examination of these animals showed that they were at the peak of the mating season. The females showed some variation. Out of a total of 18 females, 11 had greatly enlarged vaginae that were completely open, while the other seven had very marked swellings of the vagina. Of the females that had open vaginae, six had just mated (freshly formed corpora lutea). The ovaries were decidedly enlarged(4X)em.) and contained ruptured follicles, corpora lutea, atretic follicles, and mature and small follicles. The uteri were enlarged and glands were very prominent. The vaginal smear contained cornified cells and leucocytes in all four cases.

Of the other two females examined, there was evidence that one was in active oestrus. The follicles were enlarged, uteri red and enlarged with enlarged glands which contained a clear fluid. The vagina was lined with a thick layer of cornified cells, and the fluid gave a clear cornified cell smear.

The other was apparently in the latter stage of oestrus for copulation had just taken place before the operation

as shown by the presence of spermatozoa in the vagina. The vaginal smear contained cornified cells and spermatozoa in a clear viscid fluid.

From a number of measurements taken from embryos, and the date of parturition of a number of females shipped in to this laboratory from April 16 to May 6, 1931, the following copulation dates have been obtained using 28 days as the period of gestation. One mating each for April 7, 8, 11, and 12; 5 matings, April 14; 6 matings, April 15; 4 matings, April 16; one mating April 17; 14 matings April 18; 11 matings April 19; and one mating April 26.

It is evident that most outdoor matings took place in 1931 from April 14 to April 19. The four earlier matings were of females that were in exceptionally good condition. This would indicate that the condition of the animal is one of the factors which influence the date of oestrus. The last copulation date probably a result of lack of food in the early part of the breeding season.

Referring back to the calculated dates of copulation of the laboratory females, we find that they were earlier than outdoor copulations. According to calculations these laboratory copulations were approximately as follows: Jan. 10, 27 and 28 and March 23. Their period of activity was

[illegible] from records of litters born in this laboratory it was found that there was occasionally a litter born as late as one month after the regular breeding season in the outdoor female.

Males

The males received from the outdoors in April and May, 1931 were very scrotal. The scrotum was without hair, very dark, and enlarged. All the males seemed to be in splendid condition. These males and the laboratory males retained their scrotal condition until the end of May, and almost 20% of both groups were still scrotal on June 30, whereas the last animals received from the field, May 20, were very thin and non-scrotal. However, the latter may not have been a typical outdoor group as a result of improper care and handling after capture, or there could have been a scarcity of food because of dry weather.

THE EFFECT OF PITUITARY IMPLANTS

The administration of pituitary implants from rats caused a number of progressive changes to occur throughout the entire genital system.

In the ovary there was first an enlargement of the large follicles to about the size of a normally ripe follicle (0.40-0.50mm.). Ordinarily there was little blood in the follicles up to this stage, but if the implants were continued luteinization began. There seemed to be an enlargement of blood vessels in the theca interna of the large follicles. This was accompanied by a proliferation of the granulosa cells of the graafian follicle. In many cases the blood vessels ruptured, and the follicles were filled with blood. However, this did not prevent luteinization of the follicles. As the granulosa cells increased in size and number they gradually filled the entire follicle. Connective tissue cells from the theca interna were pushed in with the converging columns of granulosa cells to form a loose network around the granulosa cells. The egg was usually hemmed in by the luteinization, and ultimately resorbed. However, in some cases the follicles were found to burst allowing the egg to escape. Continued implantation caused a further enlargement of the corpora lutea, and luteinization of all the sedium follicles. The smaller ones became cystic, so that eventually the ovary became a mass of luteinized tissue.

In the uteri the changes resembled those that occurred during the coming into sexual activity. The cells of

all layers hypertrophied to cause an enlargement. The blood supply increased, and showed a dark red color. The glands underwent an enlargement, and the epithelial lining increased in thickness as was the case during the period of sexual activity.

The vagina underwent changes similar to those of the uterus, except that the chages were indicated by the condition of the external genitalia. Not only did the swellings and cells of the vaginal fluid give an index to the changes occurring in the vagina, but in the ovaries and uteri as well. These changes were characterized by a gradual enlargement of the external genitalia, accompanied by an opening of the vagina which was usually proportioned to the size of the swelling. The leucocytes were gradually replaced by the large epithelial cells in the smear, which came from the walls of the vagina, and these in turn were replaced by the cornified cells which usually persisted as long as the implantations were continued.

Johnson and Yada (1931) reported similar changes produced by implants of the pituitary gland. However, it must be added that the number of pituitary implants required to produce these progressive changes up to the great enlargement of the follicles vary directly with the stream

and the condition of the individual animal. In most cases more implants were required during the summer months when the animals were fat than at any other time. Usually the number required at this time was from seven to nine. From September until the earlier part of December the number was usually seven. During the period from January to June the number depended entirely upon the condition of the individual animal. During the stage of a slight swelling of the vagina, the number of implants required was usually three to five; while those animals with a more or less marked vaginal swelling required only one to three. Animals with open vaginae usually showed greater swellings, and luteinization of the follicles with one or two implants.

In the males, the effect was by no means so apparent. Implantation of pituitary glands for periods corresponding to those for the females produced no marked changes as in the females. However, for longer periods of implantation there were several effects noted. In the months of October and November, implantation for a period of nine days caused an enlargement of the testes and an increase in the blood supply for the seminiferous tubules. The animals implanted were several weeks earlier in coming into active spermatogenesis than were other males.

After the males were secsted, and normal spermatogenesis was taking place, implants of the pituitary glands seemed to speed up spermatogenesis in so far as one could determine. Normally there would be about 40% of the tubules containing spermatocoes or spermatids, while the others contained the spermatocyte stages. The animals that had been implanted as many as five times in the active period had the advanced stages in at least 90% of the tubules. Measurements of the tubules indicated that implantation did not cause any increase in their size.

DISCUSSION

From these findings it is evident that the reproductive cycle of the laboratory female is somewhat abnormal. The early activity of the animals, and the irregularity of the sexual activity of the females are especially striking. The lack of normal reproduction is another striking factor. Pituitary implants cause a superexcitivity of the sexual organs and their accessories in the female, but do not seem to induce reproduction. The implanted females correspond in every way to the normal animal during the period of rut, but showed no tendency to mate with normal or implanted males.

Neither did the males show any unusual excitement. Implantation of the males did not seem to affect the genital organs as it did in the females, since it was difficult to ascertain any marked effects produced by implantation of pituitary glands for periods of one to 10 days.

The fact that the animals under normal outdoor conditions are subjected to several inhibitory conditions, which are for the most part removed under laboratory conditions explains in a way this abnormality of their reproductive cycle. In the case of the females there seems to be several factors which have a great deal to do with the inhibition of sexual activity. The summer months are usually the nursing period for most females, and so soon as this period is over and while the corpora lutea still persist the animals lay in a supply of food for the long winter months of inactivity. During this period they become fattened, and the large amount of fat stored in the mesovaria and in the mesenteries is possibly partly responsible for the inhibition of sexual activity during the fall months. The winter is, of course, spent in the dormant state of hibernation for the most part. However, it is quite likely that they do not remain in hibernation for very long periods.

Under laboratory conditions hibernation does not occur. This accounts for the early activity of most of the females. One of the inhibiting factors, however, was not removed by laboratory conditions. This was the condition of fatness which existed in the normal outdoor animals during the fall months. Many of the laboratory animals retain their fat throughout the winter since there is no stimulus for hibernation. This brings many of the animals into the active period with a large quantity of stored fat. Several of these fat animals did not show an open vagina, and fatness was probably the inhibiting factor.

The males show few changes other than the early activity and their tendency to remain scrotal for a longer time than the outdoor animal. This is probably a matter of nutrition, for the outdoor male is subjected to the hot winds and a limited diet at this time of the year.

Briefly summarized, the main changes in the cycle of the laboratory female seem to be a prolongation of the activity over a longer period of time, rather than an intensified period of activity for a shorter period of time. The matter of time is evidently controlled entirely by the temperature and condition of the animals.

In the method of implanting as outlined, the question may arise as to the hypertrophy of the gonad after re-

ovul of a piece. In a number of normal controls of both sexes a piece of the gonad was removed, and after varying periods of growth there was no observable hypertrophy.

Implantation of pituitary glands did probably cause some hypertrophy as found by Engle (1928), but this was slightly more than the normal enlargement as produced by implanting normal animals, because only a short period elapsed between the time of partial gonadectomy and implantation.

CONCLUSIONS

1. The period of anoestrous is very long, lasting usually from June to January. The period of oestrus apparently may occur within the period January to June, varying in length in individual females from about two to six weeks. Reproduction in the laboratory female is rare, but evidence of four pregnancies was obtained in 1931 in animals which had been received as adults in 1930.

2. The laboratory males showed atrophied testes with lack of spermatogenesis from June 1 to December 1. Nearly all were scrotal, showing active spermatogenesis, early in January and remained in this condition until the

end of May.

3. Both outdoor females and males became sexually active later than, and remained active a shorter time than the laboratory animals. Sexually active females were found between April 7 and April 23. The males were scrotal from about April 1 to May 20.

4. Pituitary implants in the females caused follicular growth and luteinization, but not superovulation and reproduction. The number of implants required to produce maximum follicular enlargement varied from one (in females with enlarged vaginae) to nine (in animals in anoestrous).

5. Pituitary implants into males produced more active spermatogenesis in scrotal males, but had very little effect on non-scrotal males.

ACKNOWLEDGMENTS

I wish to express my sincere thanks and appreciation to Dr. George E. Johnson for his kindly advice and assistance in the choice and study of this problem.

I am also indebted to the members of the Department of Zoology for advice and criticism in the pursuit of this problem.

LITERATURE CITED

Allen, Edgar, 1922. The oestrous cycle in the mouse.
Amer. Jour. Anat., 30:297-346.

Allen, Edgar, 1926. Precocious sexual development from anterior hypophysis implants in a monkey.
Anat. Rec., 33:313-323.

Allen, Edgar, and E. A. Doisy, 1923. An ovarian hormone. Preliminary report on its localization, extraction, and partial purification, and action in test animals.
Jour. Amer. Med. Ass'n., 81:819-821.

Asdell, S. A., and F. H. A. Marshall, 1927. On the effect of the ovarian hormone in producing proestrous development in the dog and rabbit.
Proc. Roy. Soc. (London) B., 101:185-192.

Brammell, F. W. H., and A. S. Parkes, 1927. The normal ovarian cycle in relation to oestrous production.
Quart. Jour. Exper. Physiol., 18:185-198.

Brouha, L., et Simonnet, H., 1927. L'Hypophyse et la secretion interne de l'ovaire.
Compt. rend. Soc. Biol., 96:1275-1276.

Claus, Pearl E., 1931. Separation of the anterior lobe substances and study of their individual effects.
Physiol. Zool., 4(1)36-57.

Corner, G. W. , 1925. Physiology of the corpus luteum. I. The effect of early ablation of corpus luteum upon embryo and uterus.
Amer. Jour. Physiol., 86:74-81.

Cornhauser, S. I. 1930. Hematin method of staining.
Stain Tech., 1:14.

Courrier, R. , and H. Kehl, 1929. Sur la duree de l'activite luteinique pendant la gestation.
Compt. rend. Soc. Biol., 101:343-346.

Cushing, H., and E. H. Teel, 1925. Concerning the hypophyseal (pars distalis) hormones for growth and for reproductive purposes.
Amer. Jour. Physiol., 90:323-324.

Dice, L. R., 1925. "An attempt to breed cottontail rabbits in captivity."
Jour. Mammalogy, 19:225-229.

Dripe, Della, 1919. Studies on the ovary of the sparrow (Spermophilus tridecemlineatus) with special reference to the corpus luteum.
Amer. Jour. Anat., 25:117-168.

Engle, E. T., 1926. The role of the anterior pituitary in compensatory ovarian hypertrophy.
Anat. Rec., 37:275-286.

Engle, E. T., 1929. Pituitary gonadal exchanges and heterosexual ovarian grafts.
Amer. Jour. Anat., 44:121-139.

Evans, H. M. , and J. A. Long, 1921. The effect of the anterior lobe administered intraperitoneally upon growth, maturity and oestrus cycles of the rat.
Anat. Rec., 21:62-63.

Evans, H. M., and J. A. Long, 1922. Characteristic effects upon growth, oestrus and ovulation induced by the intraperitoneal administration of fresh anterior hypophyseal substance.
Proc. Nat'l. Acad. Sci., 8:38-39.

Evans, H. M. , and R. H. Cole, 1926. The oestrus cycle in the dog. I. The vaginal smear.
Cornell Vet., 16:352.

Evans, H. M. , and M. T. Simpson, 1926. Effects of anterior hypophyseal extracts on the male.
Anat. Rec. 32:206.

Evans, H. M. , and M. T. Simpson, 1927. Experimental gigantism, differential effect of anterior hypophyseal extract on normal and gonadectomized males and females.
Anat. Rec., 35:36-37.

Evans, H. M., and M. E. Simpson, 1929. A comparison of the ovarian changes produced in immature animals by implants of hypophyseal tissue and hormone from the urine of pregnant women.
Amer. Jour. Physiol. 89:381-387.

Fevold, H. L. , F. L. Hisaw, and S. L. Leonard, 1930. The gonad-stimulating and the luteinizing hormones of the anterior lobe of the hypophysis.
Anat. Rec., 47:299.

Fevold, H. L. , F. L. Hisaw, and S. L. Leonard, 1931. The gonad-stimulating and the luteinizing hormones of the anterior lobe of the hypophysis.
Amer. Jour. Physiol. 97(2):291-301.

Hammond, J. , and F. H. A. Marshall, 1930. Oestrus and pseudopregnancy in the ferret.
Proc. Royal Soc. London, 105:607-630.

Hance, F. T. , 1917. The function of mammalian chromosomes.
Anat. Rec., 12:371-388.

Harris, R. G., and J. J. Pfiffner, 1929. Extracts of corpora lutea in relation to pregnancy.
Anat. Rec.,44:205.

Hartman, C. G., 1923. The oestrus cycle of the opossum.
Amer. Jour. Anat., 32:353-392.

Hartman, C. G., 1930. Anterior lobe of the pig and the monkey ovary.
Proc. Soc. Exper. Biol. & Med., 27:338-340.

Hill, Margaret, and A. S. Parkes, 1930. Effects of anterior pituitary preparations on the anoestrous ferret.
Jour. Physiol., 69(3):xviii (Proc. Physiol. Soc.).

Johnson, G. E. , 1917. The habits of the thirteen-lined ground squirrel.
Univ. N. Dak. Quart. Jour. 7:261-271.

Johnson, G. E. , and Jeanne Challans, 1931. Ovariectomy and corpus luteum studies on rats and ground squirrels. In Press.

Johnson, O. E., and H. T. Hill, 1930. The effect of anterior pituitary extract on the developing albino mouse.
Endocrinology, 14:400-410.

Johnson, O. E., and E. D. Taylor, 1929. The effects of daily injections of bovine anterior pituitary extract upon the developing albino rat.
Physiol Zool.,2:265-301.

Johnson, O. W., and M. J. Wade, 1931. Laboratory reproduction studies on the ground squirrel, Citellus tridecemlineatus.
Biol. Bul. v. 61.

Lee, T. G., 1903. Implantation of the ovum in Sperm-ophilus tridecemlineatus(Mitchill).
Mark Anniversary Volume, pp. 417-436.

Lepine, P., 1931. Action des doses elevees d'extrait d'hypophyse sur l'aptitude du rat a la reproduction.
Compt. rend. Soc. Biol. 106:32-34.

Long, J. A., and H. M. Evans, 1922. The oestrus cycle in the rat and its associated phenomena.
Memoirs Univ. Cal., 6:1-148.

Marrian, G. F. , and A. S. Parkes, 1929. The effect of anterior pituitary preparation administered during dietary anoestrous.
Proc. Roy. Soc. B., 105:248-258.

Marshall, F. H. A. , 1904. The oestrus cycle in the common ferret.
Quart. Jour. Mic. Sci., 48:323-343.

Marshall, F. H. A. , 1922. The Physiology of Reproduction.
Longmans, Green & Co. N. Y.

Marshall, F. H. A. , and V. R. Peel, 1910. "Fatness" as a cause of sterility.
Jour. Agric. Sci., 3:383-393.

Marshall, F. H. A. , and W. A. Tood, 1923. On the ovarian factor concerned in the occurrence of oestrus.
Jour. Physiol., 58:74-80.

Meyer, R. K. , S. L. Leonard, F. L. Hisaw, and S. J. Martin, 1930. Effect of oestrin on gonad-stimulating power of the hypophysis.
Proc. Soc. Exper. Biol. & Med., 27:702-704.

Moore, Carl R. , and T. F. Gallagher, 1930. Threshold relationships of testis hormone indicators in mammals; the rat unit.
Jour. Pharmacology and Exper. Therapeutics, 40:341-350.

Moore, Carl R. , and Dorothy Price, 1930. Functional interrelations of the anterior-hypophyseal and gonad hormones.
Anat. Rec., 47:295-299.

Nelson, W. O. , J. M. Pfiffner, and H. O. Haterius, 1930. The prolongation of pregnancy by extracts of corpus luteum.
Amer. Jour. Physiol., 91:690-695.

Papanicolaou, G. N. , and N. F. Blau, 1927. Existence of a sexual rhythm and experimental induction of heat in the dog during anoestrus.
Anat. Rec., 35:47.

Parkes, A. S. , 1928. The role of the corpus luteum in the maintenance of pregnancy.
Jour. Physiol., 65:341-349.

Parkes, A. S. , and C. W. Bellerby, 1927. Studies on the internal secretions of the ovary. V. The oestrus-inhibiting function of the corpus luteum.
Jour. Physiol. 64:233-245.

Parkes, A. S. , and F. W. R. Bramwell, 1927. The causation of the anoestrous period.
Jour. Physiol., 64:388-392.

Putnam, T. J. , 1929. Separation of growth-producing hormone from that inducing premature oestrus in the anterior pituitary gland.
Arch. Surg. 18:1699-1708.

Putnam, T. J. , E. B. Benedict, and H. M. Teel, 1929. Studies in acromegaly. VIII. Experimental canine acromegaly produced by injection of anterior lobe pituitary extract.
Arch. Surg., 18:1707.

Selle, R. M. , 1922. Changes in the vaginal epithelium of the guinea pig during the oestrus period.
Amer. Jour. Anat., 30:429-450.

Shaw, W. T. , 1925. Duration of the aestivation and hibernation of the Columbian ground squirrel (Citellus columbianus) and sex relations to the same.
Ecology, 6:75-81.

Smith, P. E. , 1926. Hastening development of the female genital system by daily homoplastic pituitary transplants.
Proc. Soc. Exper. Biol. & Med., 24:131-132.

Smith, P. E. , 1927. The induction of precocious sexual maturity by pituitary homeotransplants.
Amer. Jour. Physiol., 80:114-125.

Smith, P. E. , 1930. Hypophysectomy and a replacement therapy in the rat.
Amer. Jour. Anat., 45:205-275.

Smith, P. E. , and E. T. Engle, 1927. Induction of precocious sexual maturity in the mouse by daily pituitary homeo and heterotransplants.
Proc. Soc. Exper. Biol. & Med., 24:561-562.

Smith, P. E. , and E. T. Engle, 1927. 1927. Experimental evidence regarding the role of the anterior pituitary inthe development and regulation of the genital system.
Amer. Jour. Anat., 40:159-217.

Stockard, C. R. , and G. N. Papanicolaou, 1917. The existence of a typical oestrus cycle in the guinea-pig with a study of its histological and physiological changes.
Amer. Jour. Anat., 22:225-263.

Stockard, C. R. , and G. N. Papanicolaou, 1919. The vaginal closure membrane, copulation and the vaginal plug in the guinea-pig, with further considerations of the oestrous rhythm.
Biol. Bul., 37:222-245.

Teel, H. M. , 1929. The effect of the growth principle of the hypophysis on the female genital tract: With the report of the hypertrophic changes in a case of acromegaly.
Endocrinology 13:521-526.

Wade, O. , 1927. Breeding habits and early life of the thirteen-striped ground squirrel, Citellus tridecemlineatus(Mitchill).
Jour. Mammal., 8:259-276.

Wolf, Opal Marie, 1929. Effect of daily transplants of anterior lobe of pituitary on reproduction of frog, Rana pipiens (Schreber).
Proc. Soc. Exper. Biol. & Med., 26:692-693.

Zondek, B. , und S. Aschheim, 1927. Hypophysenvorderlappen und Ovarium.
Arch. Gynäkol.,130:1-45.

Zondek, B. , und S. Aschheim, 1928. Das Hormone des Hypophysenvorderlappens.
Klin. Wochenschr., 7:831-835.

Date Due		